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REMARKS

Claims 1-5 and 8-20 are active in this application. Support for Claims 8-20 is found in Claims 1-5 and the specification as originally filed. No new matter is added by these amendments.

Applicants wish to thank Examiner Rao and Prouty for the courteous discussion granted to the Applicants' undersigned representative on March 5, 2003. In this meeting, various amendments were discussed to address the rejections under 35 U.S.C. §112 first and second paragraphs, which are believed to be reflected in the claim amendments submitted herein. Therefore, reconsideration of the rejections and allowance of all pending claims is requested.

Claims 1-5 have been rejected under 35 U.S.C. §112, first paragraph, as both not being adequately described or enabled by the specification. These rejections are traversed for the following reasons.

Amended Claims 1-5 provide an isolated DNA coding for a protein comprising an amino acid sequence shown in SEQ ID NO: 2 (see Claim 1) or an isolated DNA comprising nucleotides 557-1171 of SEQ ID NO: 1 (see Claim 2). Unquestionably these two claims are described and enabled by the specification which describes the structure of both SEQ ID NO: 1 and SEQ ID NO: 2, see the Sequence Listing.

With respect to the isolated DNA in Claim 15 the nucleotide sequence is one that hybridizes under stringent conditions to SEQ ID NO: 1, is not less than 70% homologous to SEQ ID NO: 1, and which has an activity of making the bacterium having the protein L-homoserine resistant. Provided with the nucleotide sequence in SEQ ID NO: 1 and the tools necessary to hybridize one DNA to another DNA and determine whether it has the necessary homology and activity is within the well-described knowledge available in the art. In support of this knowledge, Applicants submit herewith a selected portion from "Short Protocols in

Molecular Biology" unit 2.10, which describes hybridization analysis of DNA(third edition, Compendium of Methods From Current Protocols and Molecular Biology, Ausubel et al (eds.) John Wiley and Sons, Inc., New York). In addition, Applicants submit herewith a homology search with the rhtB sequence from *E. coli* using the BLAST and FASTA search engines as illustrative of the ability to ascertain the percent homology between two nucleotide sequences.

With respect to the written description portion of this rejection, Applicants respectfully direct the Examiner's attention to the U.S. PTO "Synopsis of Application of Written Description Guidelines" and, in particular, Example 9 (a copy is attached for reference).

In this Example a situation that is analogous to Claim 15 is presented. The conclusion is that the claim in the Example, which is similar to Claim 15 in terms of providing for a sequence which hybridizes under stringent conditions to an allowable DNA, is adequately described. Thus, Claim 15 (and the claims dependent on Claim 15) is described because "a representative number of species is disclosed , since highly stringent hybridization conditions in combination with the coding function of DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention." (Example 9 of the "Synopsis").

In light of the foregoing, Applicants respectfully request that the two rejections under 35 U.S.C. §112, first paragraph are withdrawn.

Concerning the rejection of Claim 2 under 35 U.S.C. §112, second paragraph the phrase "under stringent conditions" from Claim 2 has been removed. With respect to Claim 15, the phrase stringent conditions is defined as one which provides a sequence that is not less than 70% homologous to nucleotides 557-1171 of SEQ ID NO: 1. In light of the above discussion concerning hybridization and homology, the phrase "stringent conditions" is

readily understood and therefore is definite within the meaning of 35 U.S.C. §112, second paragraph in view of the general knowledge available in the art (see the above discussion). Withdrawal of this ground of rejection is requested.

Applicants request that the rejection under 35 U.S.C. §101 over copending application No. 09/847,392 be held in abeyance because those claims have not yet been patented.

The objections to Claims 2-5 and the rejection under 35 U.S.C. §101 are addressed by amendment.

Applicants submit that the present application is ready for allowance. Early notification of such allowance is kindly requested.

Respectfully submitted,

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SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES

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SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES

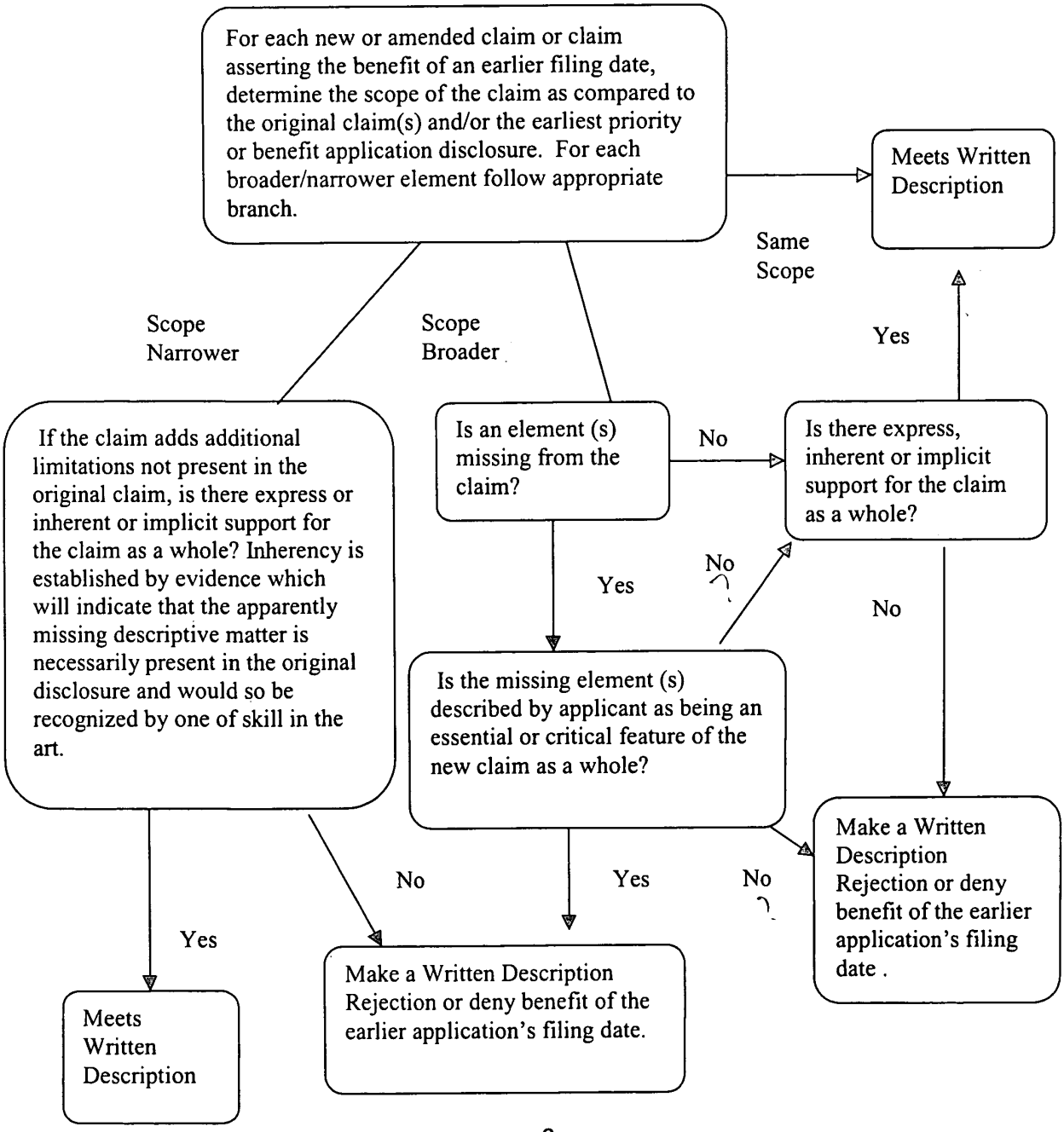
It is assumed at this point in the analysis that the specification has been reviewed and an appropriate search of the claimed subject matter has been conducted. It is also assumed that the examiner has identified which features of the claimed invention are conventional taking into account the body of existing prior art. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed. If the examiner determines that the application does not comply with the written description requirement, the examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. It should also be noted that the test for an adequate written description is separate and distinct from the test under the enablement criteria of 35 U.S.C. § 112 first paragraph. The absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, para. 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

The following examples only describe how to determine whether the written description requirement of 35 U.S.C. 112, para. 1 is satisfied. Regardless of

the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of Title 35 of the U.S. Code. Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

Written Description Amended or New Claims, or Claims Asserting the Benefit of an Earlier Filing Date

Decision Tree



Written Description
Original Claims

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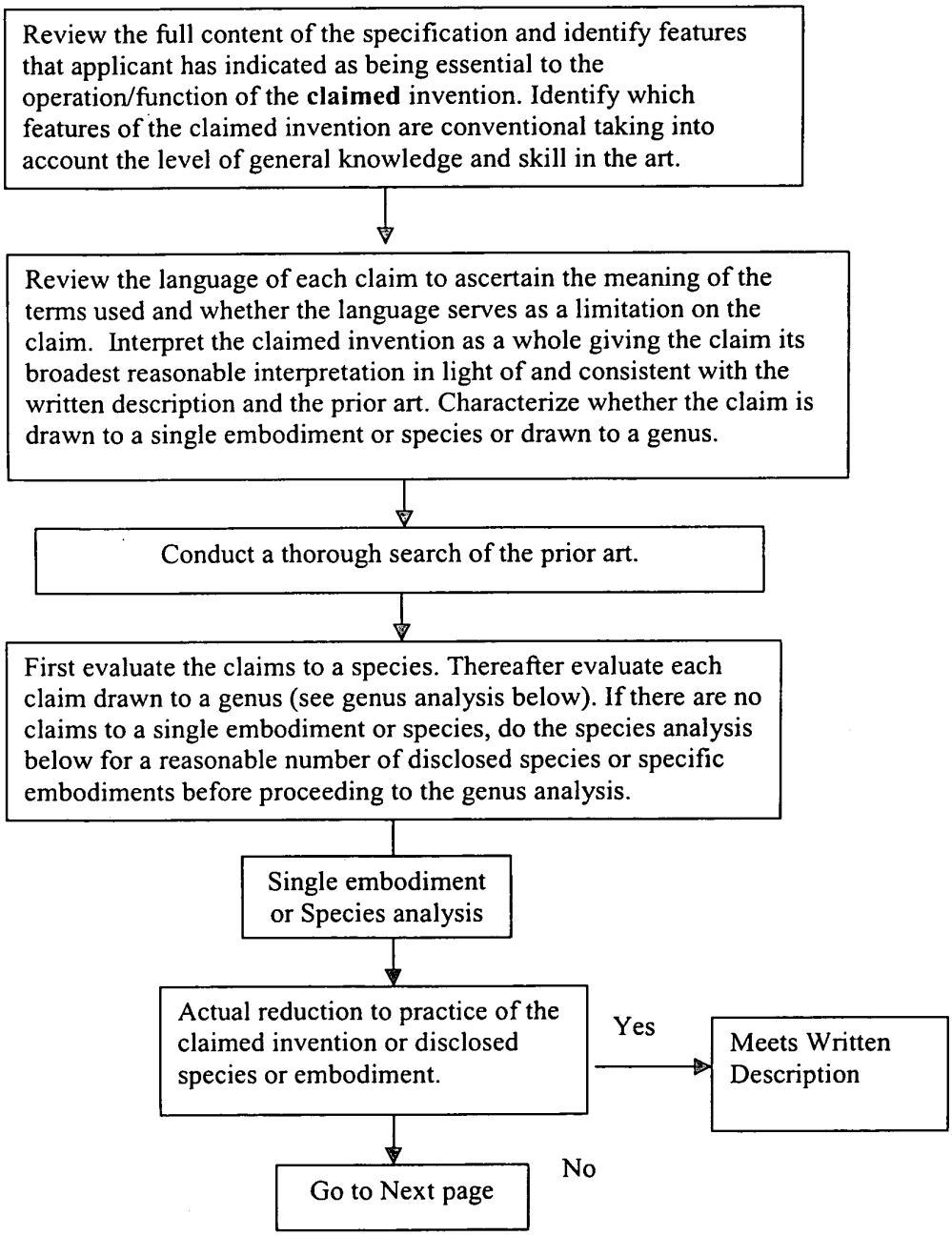

Written Description

Original Claims

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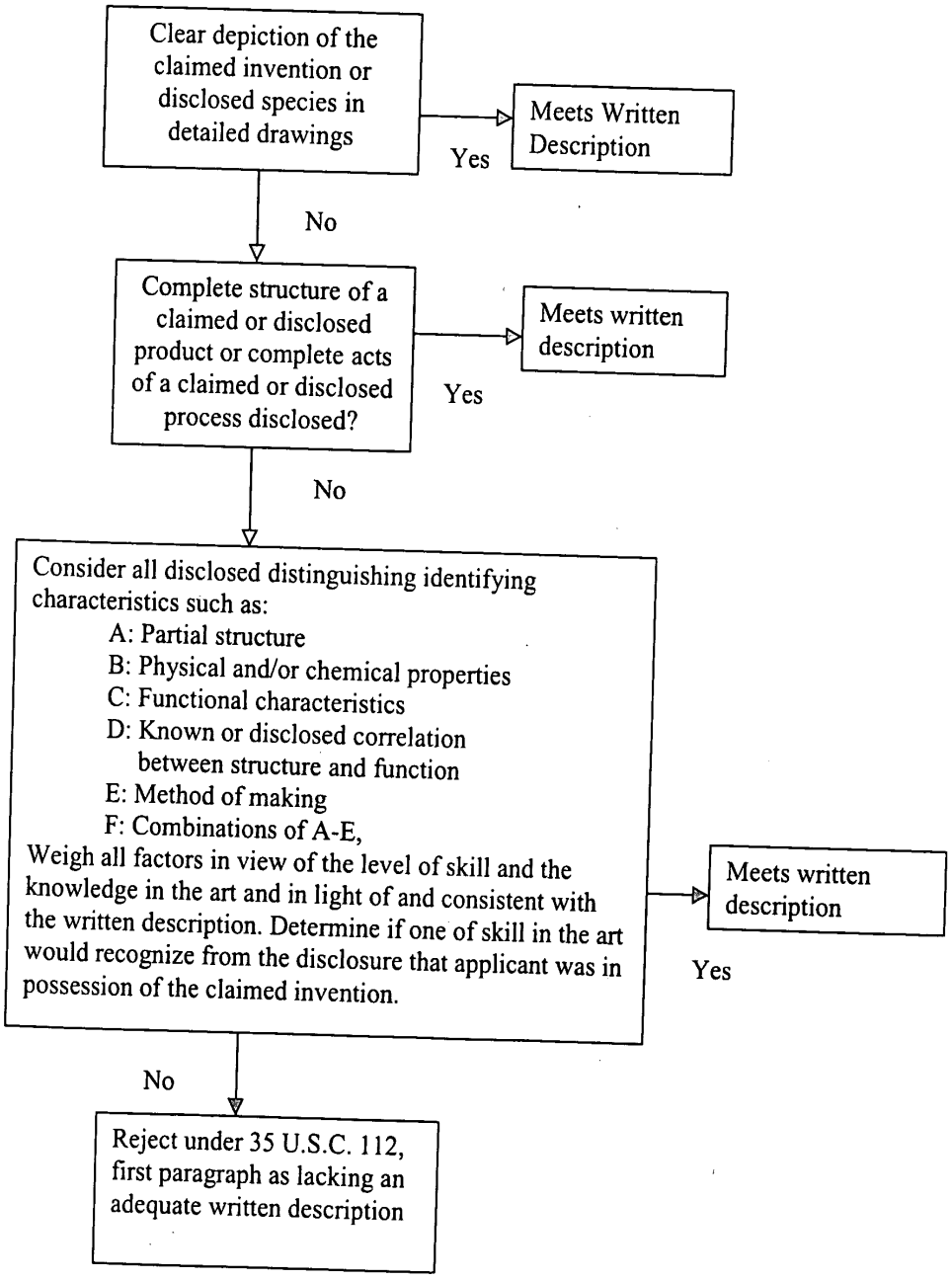

Written Description

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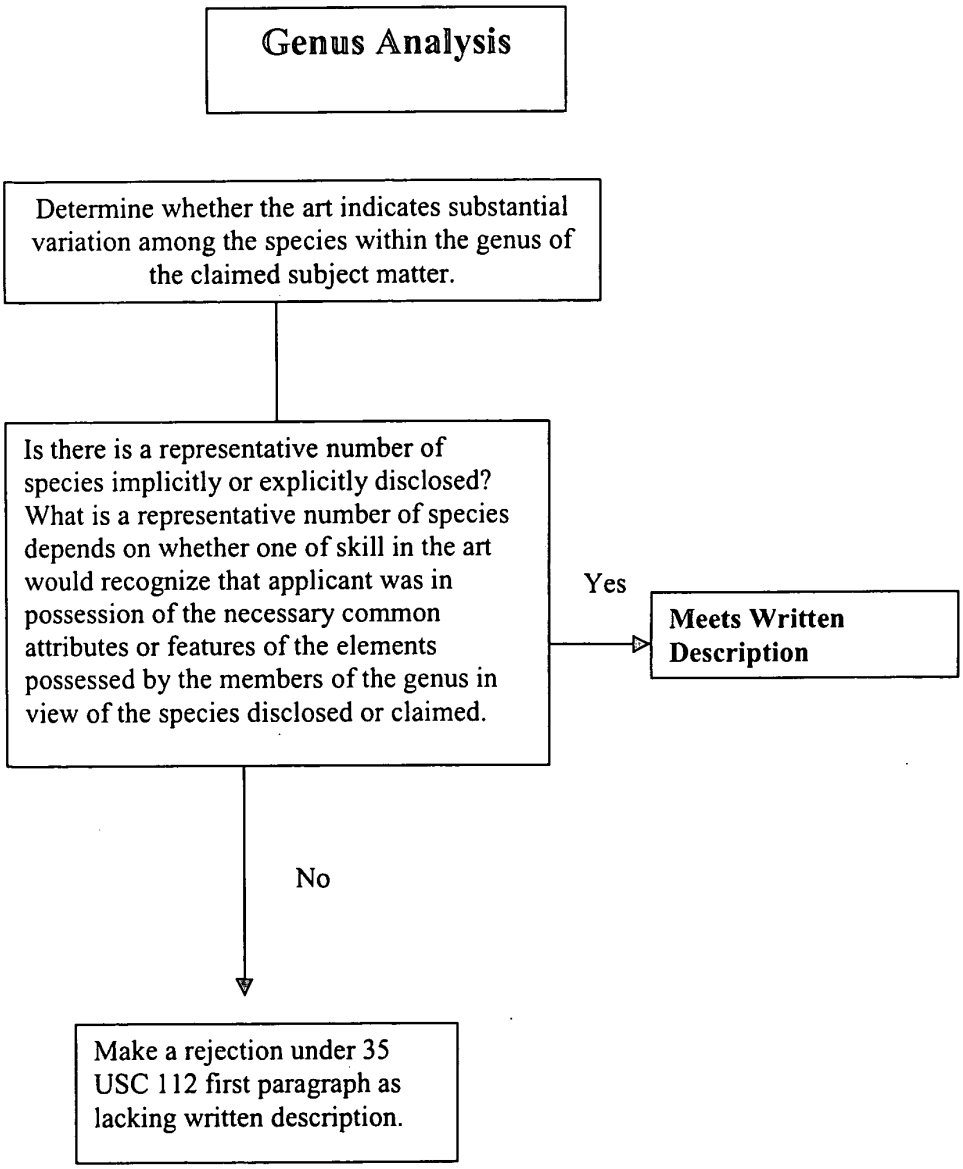

e.g. expression vectors, the necessary common attribute is the ORF (SEQ ID NO: 2).

Weighing all factors including (1) that the full length ORF (SEQ ID NO: 2) is disclosed and (2) that any substantial variability within the genus arises due to addition of elements that are not part of the inventor's particular contribution, taken in view of the level of knowledge and skill in the art, one skilled in the art would recognize from the disclosure that the applicant was in possession of the genus of DNAs that comprise SEQ ID NO: 2.

Conclusion: The written description requirement is satisfied.

Example 9: Hybridization

Specification: The specification discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. The specification includes an example wherein the complement of SEQ ID NO: 1 was used under highly stringent hybridization conditions (6XSSC and 65 degrees Celsius) for the isolation of nucleic acids that encode proteins that bind to dopamine receptor and stimulate adenylate cyclase activity. The hybridizing nucleic acids were not sequenced. They were expressed and several were shown to encode proteins that bind to a dopamine receptor and stimulate adenylate cyclase activity. These sequences may or may not be the same as SEQ ID NO: 1.

Claim:

An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1,

wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.

Analysis:

A review of the full content of the specification indicates that the essential feature of the claimed invention is the isolated nucleic acid that hybridizes to SEQ ID NO: 1 under highly stringent conditions and encodes a protein with a specific function. The art indicates that hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing.

The claim is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO: 1 and must encode a protein with a specific activity.

The search of the prior art indicates that SEQ ID NO: 1 is novel and unobvious.

There is a single species disclosed (a molecule consisting of SEQ ID NO: 1) that is within the scope of the claimed genus.

There is actual reduction to practice of the disclosed species.

Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of

skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Conclusion: The claimed invention is adequately described.

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Third Edition

A Compendium of Methods from Current Protocols in Molecular Biology

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Hybridization Analysis of DNA Blots

The principle of hybridization analysis is that a single-stranded DNA or RNA molecule of defined sequence (the "probe" which is usually labeled) can base-pair to a second DNA or RNA molecule that is immobilized and contains a complementary sequence (the "target"), with the stability of the hybrid depending on the extent of base pairing that occurs. The technique permits detection of single-copy genes in complex genomes.

BASIC PROTOCOL

HYBRIDIZATION ANALYSIS OF A DNA BLOT WITH A RADIOLABELED DNA PROBE

This protocol is suitable for hybridization analysis of Southern transfers (*UNIT 2.9A*) and dot and slot blots (*UNIT 2.9B*) with a radioactively labeled DNA probe 100 to 1000 bp in length.

Materials (see APPENDIX 1 for items with ✓)

Probe DNA labeled to a specific activity $>1 \times 10^8$ dpm/µg
✓ Aqueous prehybridization/hybridization (APH) solution, room temperature and 68°C
2× SSC/0.1% (w/v) SDS
0.2× SSC/0.1% (w/v) SDS, room temperature and 42°C
0.1× SSC/0.1% (w/v) SDS, 68°C
✓ 2× and 6× SSC

Hybridization oven (e.g., Hybridiser HB-1, Techne) *or* 68°C water bath or incubator
Hybridization tube *or* sealable bag and heat sealer

1. Wet a membrane carrying immobilized DNA in 6× SSC.
2. Place the membrane, DNA-side-up, in a hybridization tube and add ~1 ml APH solution per 10 cm^2 of membrane. Incubate 3 hr in hybridization oven with rotation at 68°C.

   For a nylon membrane, prehybridize 15 min with 68°C, prehybridization/hybridization solution. See Table 2.10.1 for other hybridization solutions and Table 2.10.2 for alternative blocking reagents.

3. Just before the end of the prehybridization incubation, denature probe DNA 10 min at 100°C. Place in ice.

Table 2.10.1 High-Salt Solutions Used in Hybridization Analysis

Stock solution	Composition
20× SSC	3.0 M NaCl/0.3 M trisodium citrate
20× SSPE[a]	3.6 M NaCl/0.2 M NaH_2PO_4/0.02 M EDTA, pH 7.7
Phosphate solution[b]	1 M $NaHPO_4$, pH 7.2[c]

[a]SSC may be replaced with the same concentration of SSPE in all protocols.

[b]Prehybridize and hybridize with 0.5 M $NaHPO_4$ (pH 7.2)/1 mM EDTA/7% SDS [or 50% formamide/0.25 M $NaHPO_4$ (pH 7.2)/0.25 M NaCl/1 mM EDTA/7% SDS]; perform moderate-stringency wash in 40 mM $NaHPO_4$ (pH 7.2)/1 mM EDTA/5% SDS; perform high-stringency wash in 40 mM $NaHPO_4$ (pH 7.2)/1 mM EDTA/1% SDS.

[c]Dissolve 134 g $NaHPO_4 \cdot 7H_2O$ in 1 liter water, then add 4 ml 85% H_3PO_4. The resulting solution is 1 M Na^+, pH 7.2.

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Table 2.10.2 Alternatives to Denhardt/Denatured Salmon Sperm DNA as Blocking Agents in DNA Hybridization[a]

Blocking agent	Composition	Storage and use
BLOTTO	5% (w/v) nonfat dried milk/0.02% (w/v) NaN_3 in H_2O	Store at 4°C; use at 4% final concentration
Heparin (porcine grade II)	50 mg/ml in 4× SSC	Store at 4°C. Use at 500 µg/ml with dextran sulfate or 50 µg/ml without
Yeast tRNA	10 mg/ml in H_2O	Store at 4°C; use at 100 µg/ml
Homopolymer DNA	1 mg/ml poly(A) or poly(C) in H_2O	Store at 4°C; use at 10 µg/ml in water; appropriate targets: poly(A) for AT-rich DNA, poly(C) for GC-rich DNA

[a]This table is based on Brown (1991) with permission from BIOS Scientific Publishers.

Table 2.10.3 Factors Influencing Hybrid Stability and Hybridization Rate[a]

Factor	Influence
A. Hybrid stability[b]	
Ionic strength	T_m increases 16.6°C for each 10-fold increase in monovalent cations between 0.01 and 0.40 M NaCl
Base composition	AT base pairs are less stable than GC base pairs in aqueous solutions containing NaCl
Destabilizing agents	Each 1% of formamide reduces the T_m by about 0.6°C for a DNA-DNA hybrid. 6 M urea reduces the T_m by about 30°C
Mismatched base pairs	T_m is reduced by 1°C for each 1% of mismatching
Duplex length	Negligible effect with probes >500 bp
B. Hybridization rate[b]	
Temperature	Maximum rate occurs at 20-25°C below T_m for DNA-DNA hybrids, 10-15°C below T_m for DNA-RNA hybrids
Ionic strength	Optimal hybridization rate at 1.5 M Na^+
Destabilizing agents	50% formamide has no effect, but higher or lower concentrations reduce the hybridization rate
Mismatched base pairs	Each 10% of mismatching reduces the hybridization rate by a factor of two
Duplex length	Hybridization rate is directly proportional to duplex length
Viscosity	Increased viscosity increases the rate of membrane hybridization; 10% dextran sulfate increases rate by factor of ten
Probe complexity	Repetitive sequences increase the hybridization rate
Base composition	Little effect
pH	Little effect between pH 5.0 and pH 9.0

[a]This table is based on Brown (1991) with permission from BIOS Scientific Publishers.

[b]There have been relatively few studies of the factors influencing membrane hybridization. In several instances extrapolations are made from what is known about solution hybridization. This is probably reliable for hybrid stability, less so for hybridization rate.

4. Pour the APH solution from the hybridization tube and replace with an equal volume of prewarmed (68°C) APH solution. Add denatured probe and incubate with rotation overnight at 68°C.

   If the specific activity of the probe is 10^8 dpm/µg, use 10 ng/ml probe; if it is 1×10^9 dpm/µg, use 2 ng/ml.

   See Table 2.10.3 for factors affecting hybrid stability and hybridization rate.

2.10

Table 2.10.4 Troubleshooting Guide for DNA Blotting and Hybridization Analysis[a]

Problem	Possible cause[b]	Solution
Poor signal	Probe specific activity too low	Check labeling protocol if specific activity is $<10^8$ dpm/μg.
	Inadequate depurination	Check depurination if transfer of DNA >5 kb is poor.
	Inadequate transfer buffer	1. Check that 20× SSC has been used as the transfer solution if small DNA fragments are retained inefficiently when transferring to nitrocellulose. 2. With some brands of nylon membrane, add 2 mM Sarkosyl to the transfer buffer. 3. Try alkaline blotting to a positively charged nylon membrane.
	Not enough target DNA	Refer to text for recommendations regarding amount of target DNA to load per blot.
	Poor immobilization of DNA	See recommendations in *UNIT 2.9A* commentary.
	Transfer time too short	See recommendations in *UNIT 2.9A* commentary.
	Inefficient transfer system	Consider vacuum blotting as an alternative to capillary transfer.
	Probe concentration too low	1. Check that the correct amount of DNA has been used in the labeling reaction. 2. Check recovery of the probe after removal of unincorporated nucleotides. 3. Use 10% dextran sulfate in the hybridization solution. 4. Change to a single-stranded probe, as reannealing of a double-stranded probe reduces its effective concentration to zero after hybridization for 8 hr.
	Incomplete denaturation of probe	Denature as described in the protocols.
	Incomplete denaturation of target DNA	When dot or slot blotting, use the double denaturation methods described in *UNIT 2.9B*, or blot onto positively charged nylon.
	Blocking agents interfering with the target-probe interaction	If using a nylon membrane, leave the blocking agents out of the hybridizadon solution.
	Final wash was too stringent	Use a lower temperature or higher salt concentration. If necessary, estimate T_m as described in *UNIT 6.4*.
	Hybridization temperature too low with an RNA probe	Increase hybridization temperature to 65°C in the presence of formamide (see Alternate Protocol).
	Hybridization time too short	If using formamide with a DNA probe, increase the hybridization time to 24 hr.
	Inappropriate membrane	Check the target molecules are not too short to be retained efficiently by the membrane type (see Table 2.9.1).

continued

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Table 2.10.4 Troubleshooting Guide for DNA Blotting and Hybridization Analysis[a], continued

Problem	Possible cause[b]	Solution
	Problems with electroblotting	Make sure no bubbles are trapped in the filter-paper stack. Soak the filter papers thoroughly in TBE before assembling the blot. Used uncharged rather than charged nylon.
Spotty background	Unincorporated nucleotides not removed from labeled probe	Follow protocols described in *UNIT 3.4*.
	Particles in the hybridization buffer	Filter the relevant solution(s).
	Agarose dried on the membrane	Rinse membrane in 2× SSC after blotting.
	Baking or UV crosslinking when membrane contains high salt	Rinse membrane in 2× SSC after blotting.
Patchy or generally high background	Insufficient blocking agents	See text for of discussion of extra/alternative blocking agents.
	Part of the membrane was allowed to dry out during hybridization or washing	Avoid by increasing the volume of solutions if necessary.
	Membranes adhered during hybridization or washing	Do not hybridize too many membranes at once (ten minigel blots for a hybridization tube, two for a bag is maximum).
	Bubbles in a hybridization bag	If using a bag, fill completely so there are no bubbles.
	Walls of hybridization bag collapsed on to membrane	Use a stiff plastic bag; increase volume of hybridization solution.
	Not enough wash solution	Increase volume of wash solution to 2 ml/10 cm^2 of membrane.
	Hybridization temperature too low with an RNA probe	Increase hybridization temperature to 65°C in the presence of formamide (see Alternate Protocol).
	Formamide needs to be deionized	Although commercial formamide is usually satisfactory, background may be reduced by deionizing immediately before use.
	Labeled probe molecules are too short	1. Use a ^{32}P-labeled probe as soon as possible after labeling, as radiolysis can result in fragmentation. 2. Reduce amount of DNase I used in nick translation (*UNIT 3.5*).
	Probe concentration too high	Check that the correct amount of DNA has been used in the labeling reaction.
	Inadequate prehybridization	Prehybridize for at least 3 hr with nitrocellulose or 15 min for nylon.
	Probe not denatured	Denature as described in the protocols.
	Inappropriate membrane type	If using a nonradioactive label, check that the membrane is compatible with the detection system.
	Hybridization with dextran sulfate	Dextran sulfate sometimes causes background hybridization. Place the membrane between Schleicher and Schuell no. 589 WH paper during hybridization, and increase volume of hybridization solution (including dextran sulfate) by 2.5%.

continued

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Table 2.10.4 Troubleshooting Guide for DNA Blotting and Hybridization Analysis[a], continued

Problem	Possible cause[b]	Solution
	Not enough SDS in wash solutions	Check the solutions are made up correctly.
Extra bands	Final wash was not stringent enough	Use a higher temperature or lower salt concentration. If necessary, estimate T_m as described in *UNIT 6.4*.
	Probe contains nonspecific sequences (e.g., vector DNA)	Purify shortest fragment that contains the desired sequence.
	Target DNA is not completely restriction digested	Check the restriction digestion (*UNIT 3.1*).
	Formamide not used with an RNA probe	RNA-DNA hybrids are relatively strong but are destabilized if formamide is used in the hybridization solution.
Nonspecific background in one or more tracks	Probe is contaminated with genomic DNA	Check purification of probe DNA. The problem is more severe when probes are labeled by random priming. Change to nick translation.
	Insufficient blocking agents	See text for of discussion of extra/alternative blocking agents.
	Final wash did not approach the desired stringency	Use a higher temperature or lower salt concentration. If necessary, estimate T_m as described in *UNIT 6.4*.
	Probe too short	Sometimes a problem with probes labeled by random priming. Change to nick translation.
Cannot remove probe after hybridization	Membrane dried out after hybridization	Make sure the membrane is stored moist between hybridization and stripping.
Decrease in signal intensity when reprobed	Poor retention of target DNA during probe stripping	1. Check calibration of UV source if cross-linking on nylon. 2. Use a less harsh stripping method (support protocol).

[a]Based on Dyson (1991).

[b]Within each category, possible causes are listed in decreasing order of likelihood.

5. Pour out the APH solution and add an equal volume of 2× SSC/0.1% SDS. Incubate with rotation 10 min at room temperature. Change the wash solution after 5 min.

   To reduce background, it may be beneficial to increase the volume of the wash solutions by 100%.

6. Replace the wash solution with an equal volume of 0.2× SSC/0.1% SDS and incubate with rotation 10 min at room temperature. Change the wash solution after 5 min (low-stringency wash).

7. If desired, carry out two 15-min moderate-stringency washes using 42°C 0.2× SSC/0.1% SDS.

8. If desired, carry out two 15-min high-stringency washes using 68°C 0.1× SSC/0.1% SDS.

9. Pour off the final wash solution, rinse the membrane in 2× SSC at room temperature, and blot excess liquid. Wrap in plastic wrap. Autoradiograph.

   Do not allow the membrane to dry out if it is to be reprobed. See Table 2.10.4 for troubleshooting.

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HYBRIDIZATION ANALYSIS OF A DNA BLOT WITH A RADIOLABELED RNA PROBE

ALTERNATE PROTOCOL

Purified RNA polymerases from bacteriophages such as SP6, T3, and T7 (*UNIT 3.8*) are very efficient at synthesizing RNA in vitro from DNA sequences cloned downstream of the appropriate phage promoter (Little and Jackson, 1987). If a radiolabeled ribonucleotide is added to the reaction mixture, the polymerase synthesizes several micrograms of uniformly labeled single-stranded RNA with specific activities $\geq 10^9$ dpm/µg. The hybridization procedure is suitable for both nitrocellulose and nylon membranes, though backgrounds may be higher with nylon.

Additional Materials (also see Basic Protocol; see APPENDIX 1 for items with ✓)

- ✓ TE buffer, pH 8.0
- ✓ Labeling buffer
- ✓ Nucleotide mix
- ✓ 200 mM dithiothreitol (DTT), freshly prepared
- 20 U/µl human placental ribonuclease inhibitor
- [α-^{32}P]UTP, 20 mCi/ml (800 Ci/mmol) or 10 mCi/ml (400 Ci/mmol)
- SP6 or T7 RNA polymerase (*UNIT 3.8*)
- ✓ RNase-free DNase I
- ✓ 0.25 M EDTA, pH 8.0
- ✓ Formamide prehybridization/hybridization (FPH) solution
- ✓ 2× SSC containing 25 µg/ml RNase A + 10 U/ml RNase T1 (*UNIT 2.13*)

1. Digest cloned DNA (see Table 2.10.5 for suitable vectors) for the sequence to be transcribed with a restriction endonuclease to linearize and introduce an endpoint for RNA synthesis. Purify the DNA by phenol extraction and ethanol precipitation and resuspend in TE buffer, pH 8.0, at a concentration of 1 mg/ml.

2. Mix the following at room temperature:

   4 µl labeling buffer
   1.5 µl nucleotide mix
   1 µl 200 mM DTT
   1 µl (20 U) human placental ribonuclease inhibitor
   2 µg purified plasmid DNA from step 1
   100 to 200 µCi [α-^{32}P]UTP
   H_2O to a final volume of 20 µl.

Table 2.10.5 Selection of Cloning Vectors Incorporating Promoters for Bacteriophage RNA Polymerases

Vector	Size (bp)	Markers[a]	Promoters
pBluescript	2950	amp, *lacZ'*	T3, T7
pGEM series	2746-3223	amp, *lacZ'*	SP6, T7
pGEMEX-1	4200	amp	SP6, T3, T7
pSELECT-1	3422	tet, *lacZ'*	SP6, T7
pSP18, 19, 64, 65	2999-3010	amp	SP6
pSP70, 71, 72, 73	2417-2464	amp	SP6, T7
pSPORT1	4109	amp, *lacZ'*	SP6, T7
pT3/T7 series	2700, 2950	amp, *lacZ'*	T3, T7
pWE15	8800	amp, neo	T3, T7
pWE16	8800	amp, dhfr	T3, T7

[a]Abbreviations: amp, ampicillin resistance; dhfr, dihydrofolate reductase; *lacZ'*, β-galactosidase α-peptide; neo, neomycin phosphotransferase (kanamycin resistance); tet, tetracycline resistance.

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3. Add 5 U of SP6 or T7 RNA polymerase. Incubate 1 hr at 40°C for SP6 polymerase or at 37°C for T7 polymerase.
4. Add 2 U RNase-free DNase I and incubate 10 min at 37°C. Stop the reaction by adding 2 µl of 0.25 M EDTA, pH 8.0.
5. Measure the specific activity of the RNA by acid precipitation and remove unincorporated nucleotides by the spin-column procedure *UNIT 3.4*. Store labeled probe up to 2 days at –20°C.

   The specific activity should be at least 7×10^8 dpm/µg, preferably $>10^9$ dpm/µg.
6. Prehybridize in FPH solution and incubate 3 hr at 42°C.
7. Replace the FPH solution with an equal volume of fresh prewarmed solution. Add the labeled probe to a concentration of 1 to 5 ng/ml and incubate overnight with rotation at 42°C.
8. Carry out two 10-min low-stringency washes in 2× SSC/0.1% at room temperature.
9. Replace the wash solution with an equal volume of 2× SSC containing 25 µg/ml RNase A + 10 U/ml RNase T1; incubate with rotation for 30 min at room temperature.
10. Carry out moderate- and high-stringency washes as desired, rinse the membrane in 2× SSC, and set up autoradiography.

SUPPORT PROTOCOL

REMOVAL OF PROBES FROM HYBRIDIZED MEMBRANES

If the DNA has been immobilized on the membrane by UV crosslinking (for uncharged nylon membranes) or by alkaline transfer (for positively charged nylon), the covalent matrix-target DNA interaction is much stronger than the hydrogen-bonded target-probe interaction, so it is possible to remove (or "strip off") the hybridized probe.

Additional Materials (also see Basic Protocol; see APPENDIX 1 for items with ✓)

✓ Mild stripping solution
✓ Moderate stripping solution
✓ 0.4 M NaOH
0.1% (w/v) SDS, 100°C

1a. *Mild treatment:* Wash the membrane in several hundred milliliters of mild stripping solution for 2 hr at 65°C.

1b. *Moderate treatment:* Wash the membrane in 0.4 M NaOH for 30 min at 45°C. Then rinse twice in several hundred milliliters of moderate stripping solution for 10 min at room temperature.

1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping.

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up autoradiography.

If signal is still seen after autoradiography, rewash using harsher conditions.

3. The membrane can now be rehybridized or dried and st red at room temperature for later use.

Reference: Dyson, N.J. 1991.

Contributor: Terry Brown

2.11

Purification of Oligonucleotides Using Denaturing Polyacrylamide Gel Electrophoresis

UNIT 2.11

BASIC PROTOCOL

This method is useful for purifying oligonucleotides because of its speed, simplicity, and high resolution. Although yields tend to be low (<50% of applied sample), the amount of material recovered is usually far in excess of that required for most molecular biology applications. This procedure is also useful for isolating small RNAs or other single-stranded polynucleotides.

Materials (see APPENDIX 1 for items with ✓)

Concentrated ammonium hydroxide
✓ 10× and 1× TBE electrophoresis buffer, pH 8
✓ 40% acrylamide/2% bisacrylamide
TEMED
Urea
✓ 10% ammonium persulfate
✓ 2× formamide loading buffer
✓ 0.3 M sodium acetate, pH 7.5
✓ TE buffer (optional)
Thin-layer chromatography (TLC) plate with fluorescent indicator (e.g., Silica Gel F-254 or IB-F)

1. Elute the synthesized oligonucleotides from the controlled-pore glass columns with concentrated ammonium hydroxide. Heat >5 hr at 55°C.

   The elution step is normally performed by the automated DNA synthesizer. The synthesized oligonucleotides used in this procedure should not contain a 5′ trityl group.

2. Transfer the sample to microcentrifuge tubes, lyophilize in a Speedvac evaporator, and resuspend the pellet in 0.2 ml water.

3. Assemble gel casting apparatus (*UNIT 2.7*). Prepare appropriate gel solution (Table 2.11.1). For a gel of 10 cm × 16 cm × 1.6 mm, mix the following in a side-arm flask:

   25.2 g urea (final concentration 7 M)
   6 ml 10 × TBE electrophoresis buffer
   40% acrylamide/2% bisacrylamide solution (desired amount)
   H_2O to 60 ml.

   Degas 10 min and add 200 µl of 10% ammonium persulfate and 30 µl TEMED. Mix and pour the gel. Allow the gel to polymerize >30 min at room temperature.

4. Remove the comb and attach the plates to the electrophoresis tank. Fill the top and bottom troughs with 1× TBE buffer.

Reference document 1

BLASTP 2.0.11 [Jan-20-2000]

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Query= YigK no description
(206 letters)

Database: /LION/data/db/fast//nrdb_1_: /LION/data/db/fast//nrdb_2_
922,638 sequences; 292,676,692 total letters

Searching.....done

Sequences producing significant alignments:

	Score (bits)	E Value
swissnew|P27847|RHTB_ECOLI Homoserine/homoserine lactone efflux...	406	e-112
swissnew|Q9L6N6|RHTB_SALTY Homoserine/homoserine lactone efflux...	373	e-102
swissnew|Q8Z3B4|RHTB_SALTI Homoserine/homoserine lactone efflux...	372	e-102
trembl|AX030175|AX030175_1 unnamed ORF; Sequence 1 from Patent ...	353	2e-96
tremblnew|AJ414159|AJ414159_46 gene: "rhtB"; product: "putative...	326	2e-88
trembl|AE000458|ECAE458_4 gene: "yigK"; product: "orf, hypothet...	271	6e-72
trembl|AE005613|AE005613_13 gene: "yigK"; Escherichia coli O15...	269	3e-71
trembl|AE004104|AE004104_3 gene: "VC0136"; product: "conserved ...	221	7e-57
trembl|AE004937|AE004937_6 gene: "PA5249"; product: "hypothetic...	175	8e-43
trembl|AP003001|AP003001_136 gene: "mlr3188"; Mesorhizobium lo...	133	2e-30
tremblnew|AL591789|SME591789_263 gene: "SMc01851"; product: "PU...	120	2e-26
trembl|AE008957|AE008957_7 gene: "rhtB"; product: "RhtB family ...	115	7e-25
trembl|AE007905|AE007905_4 gene: "AGR_pAT_516"; product: "AGR_p...	115	7e-25
trembl|AE009140|AE009140_11 gene: "rhtB"; product: "homoserine/...	111	1e-23
trembl|AE008106|AE008106_4 gene: "AGR_C_3421"; product: "AGR_C_...	111	1e-23
trembl|AP003013|AP003013_206 gene: "mlr8191"; Mesorhizobium lo...	109	4e-23
tremblnew|AL591792|SME591792_48 gene: "SMc02981"; product: "PUT...	109	5e-23
trembl|AE009276|AE009276_5 gene: "rhtB"; product: "RhtB family ...	107	1e-22
trembl|AE008336|AE008336_6 gene: "AGR_L_2738"; product: "AGR_L_...	107	1e-22
trembl|AE002037|AE002037_12 gene: "DR1999"; product: "conserved...	106	4e-22
trembl|AP003005|AP003005_190 gene: "mlr5029"; Mesorhizobium lo...	103	2e-21
trembl|AL646059|AL646059_37 gene: "RSc0418"; product: "PROBABLE...	103	3e-21
trembl|AP002995|AP002995_148 gene: "mll0653"; Mesorhizobium lo...	102	6e-21
trembl|AE009053|AE009053_1 gene: "rhtB"; product: "RhtB family ...	100	3e-20
trembl|AE008019|AE008019_1 gene: "AGR_C_1583"; product: "AGR_C_...	100	3e-20
trembl|AE004864|AE004864_7 gene: "PA4507"; product: "hypothetic...	99	4e-20
trembl|AE008180|AE008180_5 gene: "AGR_C_4838"; product: "AGR_C_...	97	3e-19
tremblnew|AE013570|AE013570_2 gene: "MM3123"; product: "hypothe...	95	8e-19
trembl|AP003012|AP003012_111 gene: "mll7642"; Mesorhizobium lo...	95	1e-18
tremblnew|AE010867|AE010867_1 gene: "MA1855"; product: "conserv...	95	1e-18
trembl|AE004079|AE004079_6 gene: "XF2730"; product: "amino acid...	94	1e-18
trembl|AP003004|AP003004_201 gene: "mll4618"; Mesorhizobium lo...	94	2e-18
trembl|AP003008|AP003008_191 gene: "mlr6177"; Mesorhizobium lo...	93	3e-18
trembl|AP001508|AP001508_152 gene: "BH0429"; Bacillus halodura...	93	4e-18
trembl|AL646057|AL646057_73 gene: "RSc0073"; product: "PROBABLE...	92	7e-18
tremblnew|AL672112|MLO672112_120 gene: "msi120"; product: "PROB...	92	9e-18
trembl|AP003012|AP003012_311 gene: "mlr7900"; Mesorhizobium lo...	91	1e-17
tremblnew|AE005687|AE005687_3 gene: "CC0126"; product: "efflux ...	91	1e-17
trembl|AL646065|AL646065_14 gene: "RSc1520"; product: "PROBABLE...	91	2e-17
trembl|AL646084|AL646084_42 gene: "RSp1321"; product: "PROBABLE...	90	2e-17
trembl|AB016260|AB016260_101 gene: "tiorf101"; Agrobacterium t...	90	4e-17
trembl|AB016260|AB016260_64 gene: "tiorf64"; Agrobacterium tum...	89	5e-17
tremblnew|AL591793|SME591793_94 gene: "SMc03827"; product: "PUT...	89	5e-17
swissnew|O05406|YRHP_BACSU Hypothetical protein yrhP.//:swiss|O...	89	6e-17
trembl|AE004221|AE004221_1 gene: "VC1421"; product: "conserved ...	89	8e-17
trembl|AE004372|AE004372_14 gene: "VCA0355"; product: "conserve...	87	2e-16
trembl|AE009620|AE009620_9 gene: "BMEI1869"; product: "HOMOSERI...	86	5e-16
tremblnew|AE011191|AE011191_72 gene: "BXB0078"; product: "amino...	85	7e-16
trembl|AL646061|AL646061_45 gene: "RSc0814"; product: "PROBABLE...	85	9e-16
trembl|AE009054|AE009054_1 gene: "rhtB"; product: "RhtB family ...	84	2e-15
trembl|AE008019|AE008019_14 gene: "AGR_C_1604"; product: "AGR_C...	84	2e-15
tremblnew|AL627271|AL627271_187 gene: "STY1851"; product: "puta...	82	6e-15
tremblnew|AE005677|AE005677_9 gene: "CC0029"; product: "efflux ...	82	6e-15
trembl|AE004269|AE004269_10 gene: "VC1939"; product: "conserved...	82	8e-15
tremblnew|AE005874|AE005874_11 gene: "CC2013"; product: "LysE f...	82	8e-15
trembl|AP003013|AP003013_244 gene: "mll8240"; Mesorhizobium lo...	82	1e-14
trembl|AE004786|AE004786_5 gene: "PA3665"; product: "hypothetic...	81	1e-14
trembl|AP002558|AP002558_210 gene: "ECs2507"; product: "hypothe...	81	2e-14

trembl|AL646058|AL646058_38 gene: "RSc0231"; product: "PUTATIVE...	81	2e-14
trembl|AE009002|AE009002_9 gene: "rhtB"; product: "RhtB family ...	81	2e-14
trembl|AE007969|AE007969_10 gene: "AGR_C_546"; product: "AGR_C_...	81	2e-14
swiss|P76249|YEAS_ECOLI Hypothetical protein yeaS.//:trembl|AX0...	81	2e-14
trembl|AP003004|AP003004_8 gene: "mll4363"; Mesorhizobium loti...	80	2e-14
tremblnew|AL591792|SME591792_226 gene: "SMc02484"; product: "PU...	80	2e-14
tremblnew|AL591783|SME591783_112 gene: "SMc00422"; product: "PU...	80	2e-14
tremblnew|AP005282|AP005282_141 gene: "Cgl2656"; product: "Puta...	80	2e-14
tremblnew|AL603646|RME603646_251 gene: "SMb21507"; product: "pu...	79	5e-14
trembl|AE008046|AE008046_1 gene: "AGR_C_2164"; product: "AGR_C_...	79	7e-14
trembl|AE005402|AE005402_13 gene: "yeaS"; product: "orf, hypoth...	79	7e-14
tremblnew|AL591783|SME591783_113 gene: "SMc00423"; product: "PU...	79	7e-14
trembl|AP001519|AP001519_10 gene: "BH3495"; product: "dihydrodi...	78	1e-13
swiss|P38102|YBF7_PSEAE Hypothetical protein PA4757.//:trembl|A...	78	1e-13
trembl|AE009457|AE009457_4 gene: "BMEI0143"; product: "THREONIN...	78	2e-13
trembl|U04992|PAARAB_3 product: "unknown"; Pseudomonas aerugin...	77	3e-13
trembl|AP003005|AP003005_160 gene: "mlr4987"; Mesorhizobium lo...	77	3e-13
tremblnew|AL591785|SME591785_187 gene: "SMc00044"; product: "PU...	77	3e-13
trembl|AE009210|AE009210_6 gene: "rhtB"; product: "RhtB family ...	75	8e-13
trembl|AE008177|AE008177_2 gene: "AGR_C_4773"; product: "AGR_C_...	75	8e-13
tremblnew|AL591783|SME591783_3 gene: "SMc02907"; product: "PUTA...	75	8e-13
trembl|AP003000|AP003000_8 gene: "mll2564"; Mesorhizobium loti...	75	1e-12
trembl|AE009738|AE009738_6 gene: "BMEII1057"; product: "TRANSPO...	73	3e-12
trembl|U93874|BSU93874_16 gene: "yrhP"; product: "YrhP"; Bacil...	73	4e-12
trembl|AF157493|AF157493_15 gene: "zm10orf7"; product: "hypothe...	73	4e-12
trembl|X67020|SCMLGA_1 gene: "mlgA"; product: "MlgA"; S.colwel...	72	9e-12
trembl|AE004589|AE004589_12 gene: "PA1620"; product: "hypotheti...	72	9e-12
tremblnew|AE005719|AE005719_4 gene: "CC0456"; product: "efflux ...	71	1e-11
tremblnew|AL049628|SCE94_12 gene: "SCE94.13"; product: "putativ...	70	3e-11
tremblnew|AL591789|SME591789_180 gene: "SMc01425"; product: "HY...	69	5e-11
trembl|AP003003|AP003003_174 gene: "mll4109"; Mesorhizobium lo...	69	8e-11
trembl|AP001517|AP001517_54 gene: "BH2932"; Bacillus haloduran...	68	1e-10
trembl|AE004718|AE004718_1 gene: "PA2916"; product: "hypothetic...	68	1e-10
trembl|AE004699|AE004699_3 gene: "PA2710"; product: "hypothetic...	68	1e-10
trembl|AP002997|AP002997_145 gene: "mll1430"; Mesorhizobium lo...	68	2e-10
trembl|AE004719|AE004719_3 gene: "PA2929"; product: "hypothetic...	67	2e-10
trembl|AE004412|AE004412_7 gene: "VCA0846"; product: "conserved...	67	3e-10
trembl|AJ311775|RFA311775_2 gene: "attX"; product: "AttX protei...	66	7e-10
trembl|AE004109|AE004109_6 gene: "VC0191"; product: "conserved ...	65	9e-10
tremblnew|AL591793|SME591793_154 gene: "SMc03887"; product: "PU...	65	9e-10
trembl|AE004657|AE004657_1 gene: "PA2306"; product: "conserved ...	64	2e-09
swissnew|P74343|YG27_SYNY3 Hypothetical protein slr1627.//:swis...	64	2e-09
trembl|AE008257|AE008257_13 gene: "AGR_L_1188"; product: "AGR_L...	64	2e-09
tremblnew|AJ414143|AJ414143_14 gene: "YPO0483"; product: "putat...	64	3e-09
trembl|AE004427|AE004427_1 gene: "VCA1000"; product: "conserved...	62	6e-09
swiss|P38101|YFIK_ECOLI Hypothetical protein yfiK.//:trembl|AX0...	62	1e-08
tremblnew|AP005274|AP005274_146 gene: "Cgl0146"; product: "Puta...	61	1e-08
trembl|AF346499|AF346499_12 product: "unknown"; Photorhabdus l...	61	2e-08
trembl|AE008712|AE008712_6 gene: "yahN"; product: "paral putati...	60	4e-08
trembl|AE005488|AE005488_5 gene: "yfiK"; product: "orf, hypothe...	60	4e-08
tremblnew|AL627266|AL627266_138 gene: "STY0397"; product: "RhtC...	60	4e-08
trembl|AE004249|AE004249_3 gene: "VC1712"; product: "conserved ...	59	5e-08
swissnew|Q9L6N7|RHTC_SALTY Threonine efflux protein.//:trembl|A...	59	7e-08
swissnew|Q8Z3B3|RHTC_SALTI Threonine efflux protein.//:tremblne...	59	7e-08
tremblnew|AP005281|AP005281_140 gene: "Cgl2344"; product: "Puta...	59	9e-08
swiss|O87005|CHPE_PSEAE Chemotactic transduction protein chpE./...	57	2e-07
trembl|U73857|ECU73857_48 unnamed ORF; Escherichia coli chromos...	56	6e-07
swissnew|P27846|RHTC_ECOLI Threonine efflux protein.//:swiss|P2...	55	1e-06
trembl|AE008820|AE008820_5 gene: "yfiK"; product: "paral putati...	55	1e-06
tremblnew|AL627275|AL627275_105 gene: "STY2838"; product: "puta...	53	3e-06
tremblnew|AL672112|HLO672112_42 gene: "msi042"; product: "PROBA...	53	4e-06
tremblnew|AJ414145|AJ414145_85 gene: "YPO0918"; product: "putat...	52	1e-05
trembl|AL646082|AL646082_70 gene: "RSp1025"; product: "PUTATIVE...	50	3e-05
tremblnew|AE000910|AE000910_1 gene: "MTH1494"; product: "conser...	48	2e-04
swissnew|Q57320|YD07_HAEIN Hypothetical protein HI1307.//:swiss...	46	4e-04
trembl|U65741|ASU65741_4 gene: "yggA"; product: "YggA"; Aeromo...	45	9e-04
swissnew|P70775|YGGA_AERSA Hypothetical protein yggA.//:swiss|P...	45	9e-04
trembl|AE008841|AE008841_6 gene: "yggA"; product: "putative LYS...	43	0.006
tremblnew|AL627277|AL627277_100 gene: "STY3222"; product: "poss...	43	0.006
tremblnew|AE010897|AE010897_6 gene: "MA2108"; product: "LysE ty...	40	0.037
swissnew|P11667|YGGA_ECOLI Hypothetical protein yggA.//:swiss|P...	39	0.064
trembl|X14436|ECFDAPGK_8 unnamed ORF; Escherichia coli fda, pgk...	39	0.084
tremblnew|AL591793|SME591793_193 gene: "SMc04404"; product: "PU...	38	0.11
trembl|AP002563|AP002563_145 gene: "ECs3794"; product: "hypothe...	38	0.14
trembl|AE004946|AE004946_10 gene: "PA5341"; product: "hypotheti...	38	0.14
trembl|AE005522|AE005522_7 gene: "yggA"; product: "orf, hypothe...	38	0.19
trembl|AF010496|AF010496_4 product: "hypothetical protein"; Rh...	37	0.25

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tremblnew:AE011957:AE011957_11 gene: "yggA"; product: "membrane...   37  0.25
trembl:AJ248287:CNSPAX05_227 product: "NADH-dehydrogenase relat...   37  0.32
trembl:AE004852:AE004852_6 gene: "PA4365"; product: "probable t...   36  0.56
tremblnew:AE010429:AE010429_1 gene: "MK1358"; product: "Putativ...   36  0.56
trembl:AB015670:AB015670_3 unnamed ORF; Bacillus sp. genes for ...   36  0.73
tremblnew:AE013520:AE013520_8 gene: "marC"; product: "Multiple ...   32  6.3
tremblnew:AJ414156:AJ414156_114 gene: "sgbK"; product: "putativ...   32  6.3

>swissnew:P27847:RHTB_ECOLI Homoserine/homoserine lactone efflux
         protein.//:swiss:P27847
         Length = 206

 Score = 406 bits (1031), Expect = e-112
 Identities = 206/206 (100%), Positives = 206/206 (100%)

Query: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
           MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG
Sbjct: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60

Query: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL 120
           LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL
Sbjct: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL 120

Query: 121 TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK 180
           TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK
Sbjct: 121 TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK 180

Query: 181 QMKALNKIFGSLFMLVGALLASARHA 206
           QMKALNKIFGSLFMLVGALLASARHA
Sbjct: 181 QMKALNKIFGSLFMLVGALLASARHA 206


>swissnew:Q9L6N6:RHTB_SALTY Homoserine/homoserine lactone efflux
         protein.//:trembl:AE008
         Length = 206

 Score = 373 bits (947), Expect = e-102
 Identities = 183/206 (88%), Positives = 195/206 (93%)

Query: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
           MT EWWFAYLLTS +LSLSPGSGAINTMTTS+NHGYRGAVASIAGLQTGL IHIVLVGVG
Sbjct: 1   MTFEWWFAYLLTSTLLSLSPGSGAINTMTTSINHGYRGAVASIAGLQTGLGIHIVLVGVG 60

Query: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL 120
           LGTLFSRS++AFE+LKWAGAAYLIWLGIQQWRAAGAIDL +LA TQSR  LF+RA+FVNL
Sbjct: 61  LGTLFSRSLLAFEILKWAGAAYLIWLGIQQWRAAGAIDLHTLAQTQSRGRLFKRAIFVNL 120

Query: 121 TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK 180
           TNPKSIVFLAALFPQFIMPQQPQL QY++LGVTTIVVD++VM GYATLAQRIA WIKGPK
Sbjct: 121 TNPKSIVFLAALFPQFIMPQQPQLAQYLILGVTTIVVDMVVMTGYATLAQRIAAWIKGPK 180

Query: 181 QMKALNKIFGSLFMLVGALLASARHA 206
           QMKALNK FGSLFMLVGALLASARHA
Sbjct: 181 QMKALNKAFGSLFMLVGALLASARHA 206


>swissnew:Q8Z3B4:RHTB_SALTI Homoserine/homoserine lactone efflux
         protein.//:tremblnew:AL
         Length = 206

 Score = 372 bits (946), Expect = e-102
 Identities = 184/206 (89%), Positives = 194/206 (93%)

Query: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
           MT EWWFAYLLTS +LSLSPGSGAINTMTTS+NHGYRGA ASIAGLQTGL IHIVLVGVG
Sbjct: 1   MTFEWWFAYLLTSTLLSLSPGSGAINTMTTSINHGYRGAAASIAGLQTGLGIHIVLVGVG 60

Query: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL 120
           LGTLFSRS+IAFE+LKWAGAAYLIWLGIQQWRAAGAIDL +LA TQSR  LF+RA+FVNL
Sbjct: 61  LGTLFSRSLIAFEILKWAGAAYLIWLGIQQWRAAGAIDLHTLAQTQSRGRLFKRAIFVNL 120

Query: 121 TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK 180
           TNPKSIVFLAALFPQFIMPQQPQL QY++LGVTTIVVD+IVM GYATLAQRIA WIKGPK
Sbjct: 121 TNPKSIVFLAALFPQFIMPQQPQLAQYLILGVTTIVVDMIVMTGYATLAQRIAAWIKGPK 180

Query: 181 QMKALNKIFGSLFMLVGALLASARHA 206
           QMKALNK FGSLFMLVGALLASARHA
```

Sbjct: 181 QMKALNKAFGSLFMLVGALLASARHA 206

>trembl|AX030175|AX030175_1 unnamed ORF; Sequence 1 from Patent
EP1013765. //:gp|AX03017
Length = 205

Score = 353 bits (895), Expect = 2e-96
Identities = 187/207 (90%), Positives = 190/207 (91%), Gaps = 3/207 (1%)

Query: 1 MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGY-RGAVASIAGLQTGLAIHIVLVGV 59
MTLEWWFAYLLTSIIL+LSPGSGAINTMTTSLNHGY G V A +TG + GV
Sbjct: 1 MTLEWWFAYLLTSIILTLSPGSGAINTMTTSLNHGYPAGGVYCWASDRTGDSYCAGWRGV 60

Query: 60 GLGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVN 119
G TLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVN
Sbjct: 61 G--TLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVN 118

Query: 120 LTNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGP 179
LTNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGP
Sbjct: 119 LTNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGP 178

Query: 180 KQMKALNKIFGSLFMLVGALLASARHA 206
KQMKALNKIFGSLFMLVGALLASARHA
Sbjct: 179 KQMKALNKIFGSLFMLVGALLASARHA 205

>tremblnew|AJ414159|AJ414159_46 gene: "rhtB"; product: "putative
homoserine/homoserine lacto
Length = 206

Score = 326 bits (826), Expect = 2e-88
Identities = 154/204 (75%), Positives = 184/204 (89%)

Query: 1 MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
MTL+WW YLLT++ILSLSPGSGAINTM+T+++HG RG VASI GLQ GLA+HIVLVGVG
Sbjct: 1 MTLDWWLTYLLTTLILSLSPGSGAINTMSTAISHGTRGVVASIGGLQLGLAVHIVLVGVG 60

Query: 61 LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL 120
LG L S+S++AFE+LKW GAAYLIWLGIQQWRAAG++DL +LA++ RR LF+RAVFVNL
Sbjct: 61 LGALVSQSLLAFEILKWLGAAYLIWLGIQQWRAAGSLDLHALANSMPRRKLFKRAVFVNL 120

Query: 121 TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK 180
TNPKSIVFLAALFPQF++PQQPQ+ QY++LG T+++VDIIVMIGYATLA RIA WIK P+
Sbjct: 121 TNPKSIVFLAALFPQFVLPQQPQVAQYLILGSTSVIVDIIVMIGYATLATRIARWIKSPQ 180

Query: 181 QMKALNKIFGSLFMLVGALLASAR 204
QMK LN+IFG LFML+GALLA+AR
Sbjct: 181 QMKLLNRIFGGLFMLIGALLATAR 204

>trembl|AE000458|ECAE458_4 gene: "yigK"; product: "orf, hypothetical
protein"; Escheri
Length = 138

Score = 271 bits (686), Expect = 6e-72
Identities = 137/138 (99%), Positives = 138/138 (99%)

Query: 69 VIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNLTNPKSIVF 128
+IAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNLTNPKSIVF
Sbjct: 1 MIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNLTNPKSIVF 60

Query: 129 LAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI 188
LAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI
Sbjct: 61 LAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI 120

Query: 189 FGSLFMLVGALLASARHA 206
FGSLFMLVGALLASARHA
Sbjct: 121 FGSLFMLVGALLASARHA 138

>trembl|AE005613|AE005613_13 gene: "yigK"; Escherichia coli O157:H7
EDL933 genome, conti
Length = 138

Score = 269 bits (680), Expect = 3e-71
Identities = 136/138 (98%), Positives = 137/138 (98%)

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Query: 69  VIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNLTNPKSIVF 128
          +IAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNLTNPKSIVF
Sbjct: 1   MIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNLTNPKSIVF 60

Query: 129 LAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI 188
           LAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI
Sbjct: 61  LAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI 120

Query: 189 FGSLFMLVGALLASARHA 206
           FGSLFMLVGALLASA HA
Sbjct: 121 FGSLFMLVGALLASAXHA 138


>trembl|AE004104|AE004104_3 gene: "VC0136"; product: "conserved
          hypothetical protein";
          Length = 205

 Score =  221 bits (558), Expect = 7e-57
 Identities = 102/204 (50%), Positives = 147/204 (72%)

Query: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
           M +  W AYLLT+++ SL+PGSG +N+++  L++G R ++ +I GLQ GLA HIVLVG+G
Sbjct: 1   MDIHVWLAYLLTAVVFSLAPGSGTVNSISNGLSYGTRHSLGAIIGLQIGLACHIVLVGIG 60

Query: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL 120
           +G L ++S +AF ++KW GAAYL+WLGIQ+WR    +   + +   S+  L ++AV +NL
Sbjct: 61  IGALVAQSALAFTLIKWIGAAYLVWLGIQKWRDRAPLTATTTSHELSQAALLRKAVLINL 120

Query: 121 TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK 180
           TNPKSIVFL ALFPQFI P +    Q++VLG+TT+ +D IVM GY  LA ++  +I+ P
Sbjct: 121 TNPKSIVFLVALFPQFIDPTRDHWPQFLVLGITTVTIDAIVMFGYTALAAQLGRYIRSPN 180

Query: 181 QMKALNKIFGSLFMLVGALLASAR 204
           M  +NK+FGS+FM  G LLA+A+
Sbjct: 181 IMTRMNKLFGSMFMGCGMLLATAK 204


>trembl|AE004937|AE004937_6 gene: "PA5249"; product: "hypothetical
          protein"; Pseudomona
          Length = 209

 Score =  175 bits (438), Expect = 8e-43
 Identities = 89/212 (41%), Positives = 129/212 (59%), Gaps = 11/212 (5%)

Query: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
           M +  WFA+ L    +SLSPG+GAI +M+  L +G+     +  GLQ GLA+ I +V  G
Sbjct: 1   MLVSTWFAFFLACWAISLSPGAGAIASMSCGLQYGFARGYWNALGLQIGLALQIAIVAAG 60

Query: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQ------R 114
           +G L + S +AF ++KW G AYL++L +QW+A          ST   R L +      R
Sbjct: 61  VGALLATSALAFSLIKWFGVAYLVYLAVRQWQAP-----PQALSTDGERPLGRPLTLVLR 115

Query: 115 AVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIAL 174
            VN +NPK+++F+ A+ PQFI P QP L QY+++G T IVVD+IVM GY  LA R+
Sbjct: 116 GFLVNASNPKAVIFMLAVLPQFIDPHQPLLAQYLIMGGTMIVVDLIVMAGYTGLAARVLR 175

Query: 175 WIKGPKQMKALNKIFGSLFMLVGALLASARHA 206
           ++ P+Q K +N+ F SLF+    LLA+ R A
Sbjct: 176 VLRSPRQQKLVNRTFASLFVGAAGLLATVRRA 207


>trembl|AP003001|AP003001_136 gene: "mlr3188"; Mesorhizobium loti
          DNA, complete genome, s
          Length = 204

 Score =  133 bits (332), Expect = 2e-30
 Identities = 76/204 (37%), Positives = 118/204 (57%), Gaps = 2/204 (0%)

Query: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
           M+LE + AY+L  I++ L PG     + S+  HG R  +A++AG Q GLAI I +VG+G
Sbjct: 1   MSLELYAAYVLACIVILVPGPTVTLIIANSIRHGTRAGLANVAGTQAGLAIMIAIVGIG 60

Query: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRRHLFQRAVFVNL 120
           L TL +    FE ++  GAAYLIW+G+Q +R+ G ++    A  + R   F + + V L
Sbjct: 61  LNTLIAGMGHWFEWVRLIGAAYLIWMGVQMFRSKGTLNADGTA-RKPRGGFFLQGLLVAL 119
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Query: 121 TNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPK 180
          +NPK++VF  A FPQFI PQ    +Q +V+G+T ++    +    YA  A R    +
Sbjct: 120 SNPKTLVFFGAFFPQFIAPQGNYSLQIVVMGLTAMIFAAMSDSTYALAAGRAGRLLSA-S 178

Query: 181 QMKALNKIFGSLFMLVGALLASAR 204
          ++K +++I GS  +  G  LA +R
Sbjct: 179 RIKLMSRISGSFLVGGGLWLAFSR 202


>tremblnew:AL591789:SME591789_263 gene: "SMc01851"; product:
          "PUTATIVE AMINO ACID EFFLUX PROTE
          Length = 211

 Score =  120 bits (299), Expect = 2e-26
 Identities = 72/209 (34%), Positives = 103/209 (48%), Gaps = 3/209 (1%)

Query: 1   MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGLAIHIVLVGVG 60
          M+ E WFA+   S +L   PG   +  ++ +L HG + A A++AG+  G    +   +G
Sbjct: 1   MSFEHWFAFAAASAVLLAIPGPTILLVISYALGHGRKIAGATVAGVALGDFTAMTASMLG 60

Query: 61  LGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLKSLASTQSRR---HLFQRAVF 117
          LG L + S   F VLKW GAAYL+WLGI+ WRA    D  S   T        +F
Sbjct: 61  LGALLATSAAVFTVLKWIGAAYLVWLGIKLWRAPVGNDSGSTVETSPAERPLRIFLHTYA 120

Query: 118 VNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIK 177
          V   NPKSI+P  A  PQF+   +P   Q  +   T +++  I    YA LA      I+
Sbjct: 121 VTALNPKSILFFVAFLPQFLDLSRPLFAQMAIFETTFLILATINAALYAWLAAAAGSTIR 180

Query: 178 GPKQMKALNKIFGSLFMLVGALLASARHA 206
            P   + +N++ GSL +  G L A  + A
Sbjct: 181 KPNIRRIVNRLGGSLLIGAGFLTAGLKRA 209
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 FASTA searches a protein or DNA sequence data bank
 version 3.3t05 March 30, 2000
Please cite:
 W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

 YigK.bd, 206 aa
 vs /LION/data/db/fast//nrdb library

343511333 residues in 1072686 sequences
 statistics extrapolated from 60000 to 1072324 sequences
  Expectation_n fit: rho(ln(x))= 5.7318+/-0.000184; mu= 1.2753+/- 0.011
 mean_var=73.7219+/-14.886, 0's: 47 Z-trim: 39  B-trim: 0 in 0/65
 Lambda= 0.1494

FASTA (3.34 January 2000) function [optimized, BL50 matrix (15:-5)] ktup: 2
 join: 36, opt: 24, gap-pen: -12/ -2, width:  16
The best scores are:                                      opt bits E(1072324)
swissnew:P27847:RHTB_ECOLI Homoserine/homoserine   ( 206) 1315  293 3.4e-78
swissnew:Q9L6N6:RHTB_SALTY Homoserine/homoserine   ( 206) 1211  270 1.9e-71
swissnew:Q8Z3B4:RHTB_SALTI Homoserine/homoserine   ( 206) 1210  270 2.2e-71
trembl:AX030175:AX030175_1 unnamed ORF; Sequence   ( 205) 1153  258 1.1e-67
trembl:AE013640:AE013640_7 gene: "rhtB"; product:  ( 206) 1068  240 3.6e-62
trembl:AE000458:ECAE458_4 gene: "yigK"; product:   ( 138)  882  199   3e-50
trembl:AE005613:AE005613_13 gene: "yigK"; Escher   ( 138)  874  198 9.9e-50
tremblnew:AE016800:AE016800_217 gene: "VV11138";   ( 207)  748  171 2.1e-41
trembl:AE004104:AE004104_3 gene: "VC0136"; produc  ( 205)  728  166 4.1e-40
trembl:AE004937:AE004937_6 gene: "PA5249"; produc  ( 209)  579  134 1.9e-30
tremblnew:AE016774:AE016774_197 gene: "PP0198"; p  ( 210)  538  125 8.9e-28
trembl:AP003001:AP003001_136 gene: "mlr3188";  Me  ( 204)  456  108 1.8e-22
tremblnew:AE016787:AE016787_102 gene: "PP3565"; p  ( 203)  452  107 3.3e-22
trembl:AL591789:SME591789_263 gene: "SMc01851"; p  ( 211)  414   99 9.9e-20
tremblnew:AE015463:AE015463_6 gene: "SO0122"; pro  ( 207)  400   96 7.9e-19
trembl:AF416331:AF416331_11 product: "RC110";  Ru  ( 208)  390   93 3.5e-18
trembl:AE008957:AE008957_7 gene: "rhtB"; product:  ( 216)  386   93 6.6e-18
trembl:AE007905:AE007905_4 gene: "AGR_pAT_516"; p  ( 234)  386   93 7.1e-18
trembl:AE009140:AE009140_11 gene: "rhtB"; product  ( 212)  384   92 8.8e-18
trembl:AE008106:AE008106_4 gene: "AGR_C_3421"; pr  ( 273)  384   92 1.1e-17
trembl:AE012886:AE012886_11 gene: "CT1267"; produ  ( 207)  380   91 1.6e-17
trembl:AL591792:SME591792_48 gene: "SMc02981"; pr  ( 211)  366   88 1.3e-16
trembl:AL646059:AL646059_37 gene: "RSc0418"; prod  ( 206)  362   87 2.3e-16
trembl:AP003013:AP003013_206 gene: "mlr8191";  Me  ( 208)  362   87 2.3e-16
trembl:AE009276:AE009276_5 gene: "rhtB"; product:  ( 210)  362   87 2.3e-16
trembl:AE008336:AE008336_6 gene: "AGR_L_2738"; pr  ( 278)  362   87   3e-16
trembl:AE002037:AE002037_12 gene: "DR1999"; produ  ( 241)  357   86 5.5e-16
trembl:AP003005:AP003005_190 gene: "mlr5029";  Me  ( 207)  354   86 7.6e-16
tremblnew:AP005954:AP005954_302 gene: "rhtB"; pro  ( 208)  350   85 1.4e-15
tremblnew:AP005963:AP005963_82 gene: "bl17736";    ( 210)  348   84 1.9e-15
tremblnew:AP005961:AP005961_273 gene: "bl17356";   ( 205)  340   83 6.1e-15
trembl:AE004864:AE004864_7 gene: "PA4507"; produc  ( 210)  340   83 6.2e-15
tremblnew:AP005961:AP005961_258 gene: "rhtB"; pro  ( 203)  333   81 1.7e-14
trembl:AP002995:AP002995_148 gene: "ml10653";  He  ( 203)  332   81   2e-14
trembl:AE008180:AE008180_5 gene: "AGR_C_4838"; pr  ( 235)  332   81 2.3e-14
trembl:AE009053:AE009053_1 gene: "rhtB"; product:  ( 203)  330   80 2.7e-14
trembl:AE008019:AE008019_1 gene: "AGR_C_1583"; pr  ( 239)  330   80 3.1e-14
tremblnew:AE016787:AE016787_161 gene: "PP3625"; p  ( 213)  329   80 3.3e-14
trembl:AE013570:AE013570_2 gene: "MM3123"; produc  ( 211)  322   79 9.2e-14
trembl:AL646057:AL646057_73 gene: "RSc0073"; prod  ( 215)  321   79 1.1e-13
trembl:AP003012:AP003012_111 gene: "mll7642";  Me  ( 208)  320   78 1.2e-13
tremblnew:AP005944:AP005944_54 gene: "rhtB"; prod  ( 210)  320   78 1.2e-13
trembl:AP003008:AP003008_191 gene: "mlr6177";  Me  ( 216)  320   78 1.3e-13
tremblnew:AE016782:AE016782_57 gene: "PP2171"; pr  ( 208)  319   78 1.4e-13
trembl:AE010867:AE010867_1 gene: "MA1855"; produc  ( 211)  318   78 1.7e-13
trembl:AE004079:AE004079_6 gene: "XF2730"; produc  ( 213)  317   78   2e-13
tremblnew:AE016785:AE016785_101 gene: "PP3025"; p  ( 206)  316   77 2.2e-13
trembl:AP001508:AP001508_152 gene: "BH0429";  Bac  ( 207)  316   77 2.2e-13
trembl:AL672112:MLO672112_120 gene: "msi120"; pro  ( 216)  315   77 2.7e-13
tremblnew:AE016811:AE016811_200 gene: "VV21051";   ( 199)  311   76 4.5e-13
trembl:AP003012:AP003012_311 gene: "mlr7900";  Me  ( 204)  310   76 5.4e-13
trembl:AL591793:SME591793_94 gene: "SMc03827"; pr  ( 213)  310   76 5.6e-13
trembl:AP003004:AP003004_201 gene: "ml14618";  Me  ( 205)  307   76 8.4e-13
trembl:AE009054:AE009054_1 gene: "rhtB"; product:  ( 214)  307   76 8.7e-13
trembl:AE008019:AE008019_14 gene: "AGR_C_1604"; p  ( 224)  307   76 9.1e-13
trembl:AE005687:AE005687_3 gene: "CC0126"; produc  ( 222)  304   75 1.4e-12
trembl:AE009620:AE009620_9 gene: "BMEI1869"; prod  ( 249)  304   75 1.6e-12
trembl:AE004221:AE004221_1 gene: "VC1421"; produc  ( 212)  302   74 1.8e-12
trembl:AL646084:AL646084_42 gene: "RSp1321"; prod  ( 206)  301   74 2.1e-12
trembl:AB016260:AB016260_64 gene: "tiorf64";  Agr  ( 229)  299   74 3.1e-12
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trembl|AB016260|AB016260_101 gene: "tiorf101"; A (204) 298 74 3.2e-12
tremblnew|AP005964|AP005964_207 gene: "blr8132"; (211) 296 73 4.5e-12
tremblnew|AE016805|AE016805_124 gene: "VV12509"; (219) 296 73 4.6e-12
trembl|AE011191|AE011191_72 gene: "BXB0078"; prod (205) 295 73 5.1e-12
trembl|AE004372|AE004372_14 gene: "VCA0355"; prod (208) 295 73 5.1e-12
trembl|AL646061|AL646061_45 gene: "RSc0814"; prod (210) 293 72 7e-12
trembl|AE005874|AE005874_11 gene: "CC2013"; produ (212) 291 72 9.5e-12
trembl|AE004786|AE004786_5 gene: "PA3665"; produc (207) 288 71 1.5e-11
tremblnew|AE016806|AE016806_33 gene: "VV12697"; p (211) 287 71 1.7e-11
trembl|AL646065|AL646065_14 gene: "RSc1520"; prod (220) 285 71 2.4e-11
trembl|AP003013|AP003013_244 gene: "mll8240"; Me (210) 284 71 2.7e-11
trembl|AE008046|AE008046_1 gene: "AGR_C_2164"; pr (210) 284 71 2.7e-11
tremblnew|AE015638|AE015638_6 gene: "SO1954"; pro (207) 281 70 4.1e-11
tremblnew|AE016787|AE016787_188 gene: "PP3652"; p (210) 280 70 4.9e-11
trembl|AE005677|AE005677_9 gene: "CC0029"; produc (210) 279 69 5.6e-11
trembl|AE014482|AE014482_1 gene: "BR1920"; produc (212) 278 69 6.6e-11
trembl|AE004269|AE004269_10 gene: "VC1939"; produ (206) 277 69 7.5e-11
trembl|AP005282|AP005282_141 gene: "Cg12656"; pro (207) 277 69 7.5e-11
trembl|AP003000|AP003000_8 gene: "mll2564"; Meso (210) 276 69 8.8e-11
trembl|AE008755|AE008755_10 gene: "yeaS"; product (212) 276 69 8.9e-11
trembl|AP003004|AP003004_8 gene: "mll4363"; Meso (213) 276 69 8.9e-11
trembl|AL591783|SME591783_113 gene: "SMc00423"; p (210) 275 69 1e-10
trembl|AP002558|AP002558_210 gene: "ECs2507"; pro (212) 275 69 1e-10
tremblnew|AE016761|AE016761_239 gene: "yeaS"; pro (212) 275 69 1e-10
swiss|P76249|YEAS_ECOLI Hypothetical protein yeaS (212) 274 68 1.2e-10
trembl|AE009002|AE009002_9 gene: "rhtB"; product: (205) 273 68 1.4e-10
tremblnew|AE016778|AE016778_192 gene: "PP1248"; p (255) 274 68 1.4e-10
trembl|AE007969|AE007969_10 gene: "AGR_C_546"; pr (224) 273 68 1.5e-10
trembl|AL646056|AL646056_38 gene: "RSc0231"; prod (211) 272 68 1.6e-10
trembl|AL591792|SME591792_226 gene: "SMc02484"; p (214) 272 68 1.6e-10
swiss|P38102|YBF7_PSEAE Hypothetical protein PA47 (216) 271 68 1.9e-10
trembl|AP003005|AP003005_160 gene: "mlr4987"; Me (211) 270 68 2.2e-10
trembl|AL591783|SME591783_112 gene: "SMc00422"; p (205) 269 67 2.5e-10
trembl|AL591785|SME591785_187 gene: "SMc00044"; p (212) 269 67 2.5e-10
trembl|AP001519|AP001519_10 gene: "BH3495"; produ (208) 268 67 2.9e-10
trembl|AE009457|AE009457_4 gene: "BMEI0143"; prod (212) 268 67 2.9e-10
trembl|AL603646|RME603646_251 gene: "SMb21507"; p (218) 268 67 3e-10
trembl|U04992|PAARAB_3 product: "unknown"; Pseud (216) 267 67 3.5e-10
trembl|AE005402|AE005402_13 gene: "yeaS"; product (212) 266 67 4e-10
trembl|AE004589|AE004589_12 gene: "PA1620"; produ (213) 259 65 1.1e-09
trembl|AE009210|AE009210_6 gene: "rhtB"; product: (204) 258 65 1.3e-09
trembl|AE008177|AE008177_2 gene: "AGR_C_4773"; pr (216) 258 65 1.3e-09
trembl|AE009738|AE009738_6 gene: "BMEII1057"; pro (205) 254 64 2.3e-09
trembl|AL939116|SCO939116_81 gene: "SCO3362"; pro (211) 252 64 3.2e-09
trembl|AL591783|SME591783_3 gene: "SMc02907"; pro (203) 248 63 5.6e-09
trembl|AE005719|AE005719_4 gene: "CC0456"; produc (208) 248 63 5.7e-09
trembl|AF157493|AF157493_15 gene: "zm10orf7"; pro (158) 244 62 8.2e-09
tremblnew|AE016808|AE016808_56 gene: "VV20059"; p (165) 244 62 8.6e-09
trembl|AL591789|SME591789_180 gene: "SMc01425"; p (197) 244 62 1e-08
trembl|X67020|SCMLGA_1 gene: "mlgA"; product: "Ml (153) 238 61 2e-08
tremblnew|AE015801|AE015801_1 gene: "SO3657"; pro (229) 234 60 5e-08
tremblnew|AE016783|AE016783_53 gene: "PP2429"; pr (194) 233 60 5.1e-08
trembl|AE004699|AE004699_3 gene: "PA2710"; produc (204) 233 60 5.3e-08
trembl|AJ311775|RFA311775_2 gene: "attX"; product (184) 232 59 5.6e-08
trembl|AE004657|AE004657_1 gene: "PA2306"; produc (205) 231 59 7.2e-08
trembl|AE004718|AE004718_1 gene: "PA2916"; produc (197) 229 59 9.4e-08
trembl|AE004412|AE004412_7 gene: "VCA0846"; produ (204) 229 59 9.6e-08
trembl|AE004719|AE004719_3 gene: "PA2929"; produc (204) 228 58 1.1e-07
trembl|AE004427|AE004427_1 gene: "VCA1000"; produ (209) 228 58 1.1e-07
tremblnew|AE016776|AE016776_181 gene: "PP0699"; p (204) 226 58 1.5e-07
trembl|AL591793|SME591793_154 gene: "SMc03887"; p (215) 226 58 1.6e-07
trembl|AP003003|AP003003_174 gene: "mll4109"; Me (204) 225 58 1.8e-07
tremblnew|AE016810|AE016810_20 gene: "VV20571"; p (210) 225 58 1.8e-07
tremblnew|AE016764|AE016764_233 gene: "yfiK"; pro (195) 224 58 2e-07
trembl|AP002997|AP002997_145 gene: "mll1430"; Me (198) 224 58 2e-07
swiss|P38101|YFIK_ECOLI Hypothetical protein yfiK (195) 223 57 2.3e-07
trembl|AE015279|AE015279_4 gene: "yfiK"; product: (195) 223 57 2.3e-07
tremblnew|AE016786|AE016786_205 gene: "PP3405"; p (209) 221 57 3.3e-07
trembl|AE004109|AE004109_6 gene: "VC0191"; produc (222) 219 57 4.6e-07
trembl|AE005488|AE005488_5 gene: "yfiK"; product: (195) 218 56 4.8e-07
tremblnew|AE015500|AE015500_10 gene: "SO0530"; pr (203) 218 56 5e-07
trembl|AE008257|AE008257_13 gene: "AGR_L_1188"; p (225) 218 56 5.4e-07
tremblnew|AE015617|AE015617_5 gene: "SO1716"; pro (197) 217 56 5.6e-07
tremblnew|AE016787|AE016787_189 gene: "PP3653"; p (204) 216 56 6.7e-07
trembl|AJ414143|AJ414143_14 gene: "YPO0483"; prod (204) 214 55 9.1e-07
trembl|AE013972|AE013972_6 gene: "y3691"; product (225) 214 55 9.9e-07
tremblnew|AE016752|AE016752_41 gene: "SE2365"; pr (221) 213 55 1.1e-06

swissnew:P74343:YG27_SYNY3 Hypothetical protein s (206) 211 55 1.4e-06
tremblnew:AP005958:AP005958_227 gene: "bll6498"; (202) 210 55 1.6e-06
trembl:AF346499:AF346499_12 product: "unknown"; (207) 209 54 1.9e-06
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trembl:AL627266:AL627266_138 gene: "STY0397"; pro (210) 207 54 2.6e-06
swissnew:O05406:YRHP_BACSU Hypothetical protein y (210) 206 54 3.1e-06
trembl:AE004249:AE004249_3 gene: "VC1712"; produc (204) 203 53 4.7e-06
trembl:AL627275:AL627275_105 gene: "STY2838"; pro (195) 202 53 5.2e-06
tremblnew:AE016810:AE016810_202 gene: "VV20771"; (187) 201 53 5.9e-06
trembl:AE008820:AE008820_5 gene: "yfiK"; product: (195) 201 53 6.1e-06
tremblnew:AE016785:AE016785_97 gene: "PP3021"; pr (204) 200 52 7.3e-06
trembl:U93874:BSU93874_16 gene: "yrhP"; product: (141) 196 52 9.7e-06
swiss:O87005:CHPE_PSEAE Chemotactic transduction (203) 195 51 1.5e-05
trembl:U73857:ECU73857_48 unnamed ORF; Escherichi (214) 194 51 1.9e-05
swiss:P75693:YAHN_ECOLI Hypothetical protein yahN (223) 194 51 1.9e-05
trembl:AE005212:AE005212_2 gene: "yahN"; product: (223) 190 50 3.5e-05
trembl:AP003009:AP003009_24 gene: "mll6377"; prod (212) 188 50 4.6e-05
tremblnew:AE016812:AE016812_131 gene: "VV21255"; (195) 187 50 4.9e-05
tremblnew:AE016786:AE016786_237 gene: "PP3438"; p (193) 186 49 5.7e-05
tremblnew:AE016756:AE016756_110 gene: "yahN"; pro (224) 183 49 0.0001
tremblnew:AE016783:AE016783_13 gene: "PP2388"; pr (208) 180 48 0.00015
trembl:Z47200:YEYFUABC_4 gene: "yfuD"; product: " (212) 179 48 0.00017
trembl:AL646082:AL646082_70 gene: "RSp1025"; prod (207) 178 48 0.0002
trembl:AE013640:AE013640_6 gene: "y0398"; product (206) 177 47 0.00023
tremblnew:AE016769:AE016769_293 gene: "c4745"; pr (206) 176 47 0.00027
swissnew:P27846:RHTC_ECOLI Threonine efflux prote (206) 175 47 0.00031
trembl:AE015396:AE015396_9 gene: "rhtC"; product: (206) 174 47 0.00036
tremblnew:AE016777:AE016777_129 gene: "PP0916"; p (204) 161 44 0.0025
trembl:AE000910:AE000910_1 gene: "MTH1494"; produ (208) 157 43 0.0046
swissnew:Q57320:YD07_HAEIN Hypothetical protein H (210) 156 43 0.0054
trembl:AP001517:AP001517_54 gene: "BH2932"; Baci (210) 153 42 0.0084
trembl:AE011957:AE011957_11 gene: "yggA"; product (208) 152 42 0.0097
trembl:AE014000:AE014000_10 gene: "y3962"; produc (192) 150 42 0.012
trembl:AJ414141:AJ414141_169 gene: "YP00181"; pro (200) 150 42 0.013
trembl:AE004946:AE004946_10 gene: "PA5341"; produ (206) 147 41 0.02
trembl:AP003002:AP003002_86 gene: "mll3530"; Mes (208) 147 41 0.02
trembl:AE010897:AE010897_6 gene: "MA2108"; produc (231) 145 41 0.03
trembl:AE004852:AE004852_6 gene: "PA4365"; produc (200) 142 40 0.042
tremblnew:AE016785:AE016785_99 gene: "PP3023"; pr (144) 140 39 0.042
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trembl:AE009161:AE009161_12 gene: "Atu2116"; prod (211) 141 40 0.051
trembl:AE008127:AE008127_12 gene: "AGR_C_3837"; p (220) 141 40 0.053
tremblnew:AP005221:AP005221_211 product: "putativ (224) 137 39 0.097
trembl:AP002999:AP002999_306 gene: "mlr2506"; Me (199) 134 38 0.14
trembl:AE013571:AE013571_6 gene: "MM3137"; produc (208) 134 38 0.14
trembl:AL646068:AL646068_47 gene: "RSc2088"; prod (200) 133 38 0.16
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trembl:AL591793:SME591793_193 gene: "SMc04404"; p (213) 133 38 0.17
trembl:AP005214:AP005214_145 product: "conserved (234) 133 38 0.18
trembl:AX063725:AX063725_1 unnamed ORF; Sequence (223) 132 38 0.2
trembl:AP005274:AP005274_146 gene: "Cgl0146"; pro (226) 132 38 0.21
trembl:AB015670:AB015670_3 unnamed ORF; Bacillus (198) 130 37 0.25
trembl:AJ248287:CNSPAX05_234 product: "Hydrogenas (499) 135 39 0.26
tremblnew:AP005960:AP005960_90 gene: "bll6893"; (200) 129 37 0.29
trembl:AE012410:AE012410_7 gene: "yggA"; product: (208) 128 37 0.35
trembl:AE010429:AE010429_1 gene: "MK1358"; produc (205) 127 37 0.4
trembl:AE006110:AE006110_4 gene: "PM0727"; produc (214) 127 37 0.42
trembl:AE013520:AE013520_8 gene: "marC"; product: (212) 126 37 0.48
trembl:AE004305:AE004305_8 gene: "VC2352"; produc (418) 126 37 0.86
tremblnew:AE015565:AE015565_5 gene: "SO1214"; pro (432) 125 36 1
tremblnew:AE015725:AE015725_3 gene: "SO2865"; pro (206) 119 35 1.3
tremblnew:AE016800:AE016800_48 gene: "VV10946"; p (223) 119 35 1.4
tremblnew:AE015521:AE015521_9 gene: "SO0765"; pro (234) 117 35 2

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; mp_ver: 33t05
; mp_argv: fasta33_t -E 10 -b 200 -T 4 -Q -H -m 10 -O YigK.fasta_nrdb YigK.bd
/LION/data/db/fast//nrdb
; pg_name: FASTA
; pg_ver: 3.34 January 2000
; pg_matrix: BL50 (15:-5)
; pg_gap-pen: -12 -2
; pg_ktup: 2
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; pg_cgap: 36

; mp_extrap: 60000 1072324
; mp_stats: Expectation_n fit: rho(ln(x))= 5.7318+/-0.000184; mu= 1.2753+/- 0.011
mean_var=73.7219+/-14.886, 0's: 47 Z-trim: 39 B-trim: 0 in 0/65 Lambda= 0.1494
; mp_KS: 0.0000 (N=0) at 45468
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; fa_opt: 1315
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; fa_bits: 292.7
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SLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVL
GVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLVGALL
ASARHA
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SLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVL
GVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLVGALL
ASARHA
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; fa_opt: 1211
; fa_z-score: 1423.4
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AIHIVLVGVGLGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLK
SLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVL
GVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLVGALL
ASARHA
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TLAQTQSRGRLFKRAIFVNLTNPKSIVFLAALFPQFIMPQQPQLAQYLIL
GVTTIVVDMVVMTGYATLAQRIAAWIKGPKQMKALNKAFGSLFMLVGALL
ASARHA
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; fa_init1: 1210
; fa_opt: 1210
; fa_z-score: 1422.2
; fa_bits: 270.1
; fa_expect: 2.2e-71
; sw_score: 1210
; sw_ident: 0.893
; sw_overlap: 206
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; al_start: 1
; al_stop: 206
; al_display_start: 1
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AIHIVLVGVGLGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLK
SLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVL
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KSLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIV
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LASARHA
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DSYCAGWRGVG--TLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDL
KSLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIV
LGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLVGAL
LASARHA
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lacto
; fa_frame: f
; fa_initn: 1068
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; fa_opt: 1068
; fa_z-score: 1256.8
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SLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVL
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ALANSMPRRKLFKRAVFVNLTNPKSIVFLAALFPQFVLPQQPQVAQYLIL
GSTSVIVDIIVMIGYATLATRIARWIKSPQQMKLLNRIFGGLFMLIGALL
ATARKV
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; fa_frame: f
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; sw_score: 882
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; sw_overlap: 138
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; sq_type: p
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; al_stop: 206
; al_display_start: 39
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QQWRAAGAIDLKSLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIM
PQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI
FGSLFMLVGALLASARHA
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; al_start: 1
; al_stop: 138
; al_display_start: 1
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FGSLFMLVGALLASARHA
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conti
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; fa_opt: 874
; fa_z-score: 1033.5
; fa_bits: 197.6
; fa_expect: 9.9e-50
; sw_score: 874
; sw_ident: 0.986
; sw_overlap: 138
>YigK ..
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; sq_offset: 1
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; al_start: 69
; al_stop: 206
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PQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI
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PQQPQLMQYIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKI
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; fa_bits: 170.5
; fa_expect: 2.1e-41
; sw_score: 748
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; sw_overlap: 204
>YigK ..
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VLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLVGA
LLASARHA
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; al_display_start: 1
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TASQQHAISSTALLRKAVLINLTNPKSIVFLVALFPQFIDPSQDQVTQLA
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LLAAAKS
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; fa_init1: 697
; fa_opt: 728
; fa_z-score: 860.9
; fa_bits: 166.2
; fa_expect: 4.1e-40
; sw_score: 728
; sw_ident: 0.500
; sw_overlap: 204
>YigK ..
; sq_len: 206
; sq_offset: 1
; sq_type: p
; al_start: 1
; al_stop: 204
; al_display_start: 1
MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGL
AIHIVLVGVGLGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDLK
SLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIVL
GVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLVGALL
ASARHA
>trembl|AE004104|AE004104_3 ..; sq_len: 205
; sq_type: p
; al_start: 1
; al_stop: 204
; al_display_start: 1
MDIHVWLAYLLTAVVFSLAPGSGTVNSISNGLSYGTRHSLGAIIGLQIGL

ACHIVLVGIGIGALVAQSALAFTLIKWIGAAYLVWLGIQKWRDRAPLTAT
TTSHELSQAALLRKAVLINLTNPKSIVFLVALFPQFIDPTRDHWPQFLVL
GITTVTIDAIVMFGYTALAAQLGRYIRSPNIMTRMNKLFGSMFMGCGMLL
ATAKA
>>trembl|AE004937|AE004937_6 gene: "PA5249"; product: "hypothetical protein";
Pseudomona
; fa_frame: f
; fa_initn: 561
; fa_init1: 292
; fa_opt: 579
; fa_z-score: 687.2
; fa_bits: 134.1
; fa_expect: 1.9e-30
; sw_score: 579
; sw_ident: 0.420
; sw_overlap: 207
>YigK ..
; sq_len: 206
; sq_offset: 1
; sq_type: p
; al_start: 1
; al_stop: 206
; al_display_start: 1
MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGYRGAVASIAGLQTGL
AIHIVLVGVGLGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAG-AIDL
KSLASTQSRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQYIV
LGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLVGAL
LASARHA
>trembl|AE004937|AE004937_6 ..
; sq_len: 209
; sq_type: p
; al_start: 1
; al_stop: 207
; al_display_start: 1
MLVSTWFAFFLACWAISLSPGAGAIASMSCGLQYGFARGYWNALGLQIGL
ALQIAIVAAGVGALLATSALAFSLIKWFGVAYLVYLAVRQWQAPPQALST
DGERPLGRPLTLVLRGFLVNASNPKAVIFMLAVLPQFIDPHQPLLAQYLI
MGGTMIVVDLIVMAGYTGLAARVLRVLRSPRQQKLVNRTFASLFVGAAGL
LATVRRAPL
>>tremblnew|AE016774|AE016774_197 gene: "PP0198"; product: "transporter, LysE family";
Pseudo
; fa_frame: f
; fa_initn: 495
; fa_init1: 264
; fa_opt: 538
; fa_z-score: 639.4
; fa_bits: 125.3
; fa_expect: 8.9e-28
; sw_score: 538
; sw_ident: 0.381
; sw_overlap: 210
>YigK ..
; sq_len: 206
; sq_offset: 1
; sq_type: p
; al_start: 1
; al_stop: 206
; al_display_start: 1
MTLEWWFAYLLTSIILSLSPGSGAINTMTTSLNHGY-RGAVASIAGLQTG
LAIHIVLVGVGLGTLFSRSVIAFEVLKWAGAAYLIWLGIQQWRAAGAIDL
KSLASTQ---SRRHLFQRAVFVNLTNPKSIVFLAALFPQFIMPQQPQLMQ
YIVLGVTTIVVDIIVMIGYATLAQRIALWIKGPKQMKALNKIFGSLFMLV
GALLASARHA
>tremblnew|AE016774|AE016774_197 ..
; sq_len: 210
; sq_type: p
; al_start: 1
; al_stop: 208
; al_display_start: 1
MSMEVWLGFFAACWVISLSPGAGAIASMSSGLQYGFWRGYWNAL-GLQLG
LIMQIAIIAAGVGAVLAASATAFQVIKWFGVGYLVYLAYKQWRAL-PMDM
SDESGVRPIGKPLSLVFRGFLVNISNPKALVFMLAVLPQFLNPHAPLLPQ
YVAITVTMVTVDLLVMAGYTGLASHVLRMLRTPKQQKRLNRTFAGLFIGA
ATFLATLRRAPV